Formylation of Iron(III) Porphyrins and Chlorins of Natural Origin

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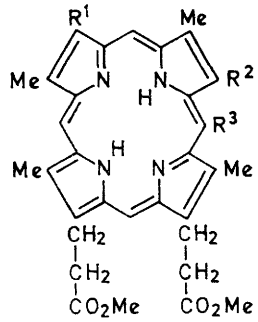
Iron(III) porphyrins and chlorins are convenient starting materials for the preparation of labile formylated derivatives by the Vilsmeier procedure. Porphyrins and chlorins bearing vinyl substituents yield 3-oxoprop-1-enyl derivatives. Both *cis*- and *trans*-isomers are formed, the latter predominating. Relative reactivities appear to be vinyl > *meso*-proton > β-proton. The n.m.r. and visible spectra of the products of formylation of deuteroporphyrin IX, mesoporphyrin IX, protoporphyrin IX, chlorin-e_6 and isochlorin-e_4 methyl esters are compared with the spectra of other formylporphyrins and chlorins.

FORMYLPORPHYRINS of known structure occur naturally as their iron complexes in the prosthetic groups of cytochrome oxidase and chlorocruorin;[1] chlorophylls *b* and *d* are the magnesium complexes of formylchlorins.[2] The prosthetic groups of lactoperoxidase and myeloperoxidase also appear to be iron porphyrins of unknown structure bearing electrophilic substituents.[3-5] A recent suggestion[6] that the prosthetic group of myeloperoxidase has a conjugated system similar to that of an iron chlorin led to the initiation of the present work as a search for spectroscopic models for this enzyme. The shift in the spectrum of the haemochrome of myeloperoxidase towards shorter wavelengths on treatment with pyridine and alkali suggested that an electron-withdrawing substituent might be located on a *meso*-carbon atom. Steric repulsion would account for ready deformylation.

Formylation of copper(II) porphyrins at the *meso*-positions has been reported by Inhoffen and his coworkers;[7] a similar reaction of nickel(II) aetioporphyrin I has been noted by Johnson and Oldfield.[8] Preliminary work with copper(II) and nickel(II) derivatives of protoporphyrin IX dimethyl ester (Ia) indicated that reaction with sulphuric acid, required for the removal of copper or nickel after formylation, caused loss of the formyl group as well as sulphonation. For this reason the use of more readily decomposable metal complexes was investigated. Manganese(III), cobalt(II), iron(III), nickel(II), and copper(II) protoporphyrin IX dimethyl esters all showed a bathochromic shift in their spectra after treatment with dimethylformamide and phosphoryl chloride. The order of the shift is consistent with the introduction of formyl substituents. Zinc(II) protoporphyrin IX dimethyl ester decomposed when heated in the formylating mixture to yield the metal-free ligand. Since iron(II) may be removed from porphyrin complexes by treatment with dilute acids at room temperature the use of haemins as starting materials was further investigated.

Protohaemin dimethyl ester reacted rapidly with dimethylformamide and phosphoryl chloride at 100°. After removal of iron, two products, characterised by rhodo-type spectra, could be separated by chromatography on alumina. The mass spectra of the compounds suggested that they were mono- and di-formylprotoporphyrins IX. Both showed the presence of all four *meso*-protons in their n.m.r. spectra and in addition two signals at slightly higher field (τ 0·36 and 0·65). In the case of the monoformyl derivative one proton was accounted for in the two signals, whereas in that of the diformyl derivative two protons were present. In the spectrum of the latter compound the signals corresponding to the vinyl groups were moved downfield and corresponded to only four protons. In the case of monoformylprotoporphyrin dimethyl ester only one vinyl group was affected in this way. Confirmation that the products had been formed by formylation at the terminal methylene of the vinyl substituent was found in the n.m.r. spectrum of bis(hydroxymethyl)protoporphyrin, formed by sodium borohydride reduction of the diformyl product. The former compound showed two



(I)

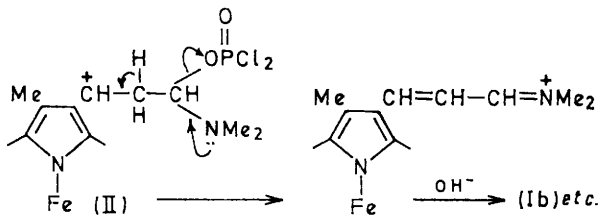
	R^1	R^2	R^3
a;	$CH=CH_2$	$CH=CH_2$	H
b;	CH=CH–CHO	$CH=CH_2$	H
c;	CH=CH–CHO	CH=CH–CHO	H
d;	H	H	CHO
e;	CHO	H	CHO
f;	Et	Et	H

clear doublets at τ 4·34 and 4·93 in the position expected for allylic methylene groups adjacent to an oxygen atom. The higher-field doublet was the more intense. The

[1] J. E. Falk, 'Porphyrins and Metalloporphyrine,' Elsevier, Amsterdam, 1964, p. 94.
[2] S. Aronoff in 'The Chlorophylls,' ed. L. P. Vernon and G. S. Seely, Academic Press, New York, 1966, p. 10.
[3] D. B. Morell and P. S. Clezy, *Biochim. Biophys. Acta*, 1963, **71**, 157.
[4] J. Schultz and H. W. Shmukler, *Biochemistry*, 1964, **3**, 1234.
[5] N. Newton, D. B. Morell, L. Clarke, and P. S. Clezy, *Biochim. Biophys. Acta*, 1965, **96**, 476.
[6] D. B. Morell, Y. Chang, I. Hendry, A. W. Nichol, and P. S. Clezy in 'Structure and Function of Cytochromes,' ed. K. Okunuki, M. D. Kamen, and I. Sekuzu, University of Tokyo Press, 1968, p. 563.
[7] H. H. Inhoffen, J. Fuhrhop, H. Voight, and H. Brockmann, *Annalen*, 1966, **695**, 133; H. Brockmann, K. Bliesener, and H. H. Inhoffen, *ibid.*, 1968, **718**, 148.
[8] A. W. Johnson and D. Oldfield, *J. Chem. Soc.* (*C*), 1966, 794.

unequal intensity of the two doublets suggested that the separation in chemical shift was caused by greater deshielding in the *cis*-isomer than in the *trans*-isomer. In the spectra of both the mono- and the di-formylporphyrin a difference in intensity between the two aldehyde proton signals occurred. In each case the *trans*-proton signal (higher field) was about twice as intense as the *cis*-proton signal. The two products are thus formulated as 2(or 4)-(3-oxoprop-1-enyl)-4(or 2)-vinyldeuteroporphyrin IX dimethyl ester (Ib) and 2,4-bis-(3-oxoprop-1-enyl)deuteroporphyrin IX dimethyl ester (Ic). The 3-oxoprop-1-enyl group may be formed by elimination of a proton from the carbonium ion (II). Solvation of this ion may explain the large amount of polar by-products which remained adsorbed on alumina during the purification of the formylated products. A similar reaction has recently been reported in the nitration of protohaemin, which yields 1-hydroxy-2-nitroethyl substituents.[9]

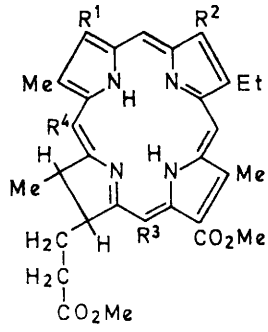
Formylation of deuterohaemin dimethyl ester occurred much less easily than that of protohaemin. In contrast to the wide range of products obtained by Inhoffen and his co-workers in the formylation of copper(II) deuteroporphyrin dimethyl ester,[7] deuterohaemin yielded only β-*meso*-formyldeuteroporphyrin dimethyl ester (Id) and 2,β-diformyldeuteroporphyrin dimethyl ester (Ie) as crystalline products. The structural assignments were based on a comparison of the visible and n.m.r. spectra of the products with those reported by Brockmann and his co-workers.[7] If the greater reactivity of the copper complex is due to an increase in the electron density in the porphyrin ring caused by π-donation from the non-bonding *d* orbitals of the metal atom, differences in the electron density between the *meso*-positions and the β-positions of the pyrrole rings may well be less in the copper(II) complex than in the haemin, resulting in a greater complexity of products from the former metal complex. Small amounts of 2(and 4)-formyldeuteroporphyrin IX dimethyl ester and 2,4-diformyldeuteroporphyrin IX dimethyl ester were, however, detected in the mother liquors of the two crystalline products by t.l.c.



Mesohaemin dimethyl ester was also formylated under the Vilsmeier conditions although it was considerably less reactive than deuterohaemin. The n.m.r. spectrum of the product revealed a low-field signal at τ −2·45 as well as only three methine protons. An upfield shift of 0·54 p.p.m. in one of the ethyl proton signals compared with mesoporphyrin IX dimethyl ester (If), as well as a difference in the position of the two propionyl methoxy-group signals suggested that the product was formylated at the β-position. The reduction in ring current due to distortion of the porphyrin ring was paralleled by a decrease in the intensity and a broadening of the bands in the visible spectrum, and by expulsion of carbon monoxide in the mass spectrum to give a base peak of $M - 28$ units. A similar fragmentation was also shown in the mass spectrum of β-*meso*-formyldeuteroporphyrin dimethyl ester; however, in this case the $M - 28$ peak was much less prominent.

A recent report[10] of the distribution of isomers formed by coupled oxidation of deuterohaemochrome and hydrazine indicates that the β-position of this compound is also more reactive to electrophilic reagents. The isolation of biliverdin IXβ from an analogous reaction with protohaemochrome[11] may indicate a similar electronic distribution in protoporphyrin although in view of Rüdiger's results on protohaemochrome,[10] the the separation of biliverdin IXβ may be due only to its lower solubility.

In contrast to deuterohaemin and mesohaemin, which required reaction times of several hours, the iron(III) chlorins reacted rapidly with dimethylformamide and phosphoryl chloride. After heating the iron complex of chlorin-e_6 trimethyl ester (IIIa) with the formylating mixture for 90 sec. two compounds were formed, with molecular ions in the mass spectrum corresponding to mono- and di-formyl derivatives of chlorin-e_6 trimethyl ester. The monoformyl derivative showed two signals of unequal intensity corresponding to one proton at τ −0·13 and −0·01. A displacement of the vinyl



(III)

	R^1	R^2	R^3	R^4
a;	$CH=CH_2$	Me	$CH_2{\cdot}CO_2Me$	H
b;	CH=CH–CHO	Me	$CH_2{\cdot}CO_2Me$	H
c;	CH=CH–CHO	Me	$CH_2{\cdot}CO_2Me$	CHO
d;	$CH=CH_2$	Me	CHO	H
e;	$CH=CH_2$	CHO	$CH_2{\cdot}CO_2Me$	H
f;	$CH=CH_2$	Me	H	H
g;	$CH=CH_2$	CN	$CH_2{\cdot}CO_2Me$	H

proton signals analogous to that found in the n.m.r. spectra of the formylprotoporphyrins allowed the assignment of the structure 2-desvinyl-2-(3-oxoprop-1-enyl)-

[9] M. Z. Atassi, *Biochim. Biophys. Acta*, 1969, **177**, 663.
[10] W. Rüdiger in 'Porphyrins and Related Compounds,' ed. T. W. Goodwin, Academic Press, London, 1968, p. 121.
[11] A. W. Nichol and D. B. Morell, *Biochim. Biophys. Acta*, 1969, **184**, 173.

TABLE 1

N.m.r. signals of some chlorins and their *meso*-substituted derivatives in deuteriochloroform

	CHO	*meso*- and 6-Proton	Vinyl or substituted vinyl CH=CHR (m)		OMe 6	OMe γ	OMe 7	7- and 8-Protons	β-Me 1	β-Me 3	β-Me 5	β-Me 8(d)	Methylenes γ	Methylenes 7′	$CH_3{\cdot}CH_2$ (t) (and 7″-CH_2)
Isochlorin-e_4 dimethyl ester		0·28 (α,β) 1·13 (δ) 1·17 (δ)	1·89	3·84		6·29	6·41	5·49	6·41	6·70	6·53	8·29	4·62	7·69	8·31
Chlorin-e_6 dimethyl ester		0·40 (β) 0·60 (α) 1·29 (δ)	2·17	3·94	5·77	6·26	6·41	5·60	6·66	6·88	6·47	8·27	4·71	7·72	8·36
Rhodin-g_7 trimethyl ester	−1·05	−0·27 (α) 0·43 (β) 1·37 (δ)	2·07	3·82	5·74	6·22	6·37	5·68	6·66		6·52	8·24	4·76	7·67	8·29
3-Cyano-3-desformylrhodin-g_7 trimethyl ester		0·59 (β) 0·73 (α) 1·35 (δ)	2·36	4·02	5·74	6·21	6·34	5·69	6·79		6·56	8·22	4·73	7·61	8·29
2-Desvinyl-2-(3-oxoprop-1-enyl)isochlorin-e_4 dimethyl ester	0·12(1/3) *cis* 0·23(2/3) *trans*	0·40 (α) 0·66 (β) 1·19 (δ) 1·21 (δ)	1·53(d) (*J* 17 Hz)	3·12		6·27	6·44	5·45	6·38	6·82	6·69	8·26	4·67	7·67	8·31
2-Desvinyl-2-(3-oxoprop-1-enyl)chlorin-e_6 trimethyl ester	−0·13(1/3) *cis* −0·01(2/3) *trans*	0·32 (α) 0·49 (β) 1·13 (δ)	1·35(d) (*J* 17 Hz)	2·87	5·72	6·22	6·42	5·60	6·35	6·78	6·53	8·25	4·70	7·68	8·34
2-Desvinyl-δ-formyl-2-(3-oxoprop-1-enyl)-chlorin-e_6 trimethyl ester	−1·59 δ −0·11 *cis* 0·00 *trans*	0·67 (α) 0·73 (β)	1·38(d) (*J* 17 Hz)	2·54	5·76	6·19	6·31	4·80 5·69	6·63	6·93	6·75	8·67	4·98	7·53	8·38
Purpurin-5 dimethyl ester	−1·51	0·49 (β) 0·78 (α) 1·50 (δ)	2·23	3·89	5·81		6·41	5·01 5·75	6·74	6·97	6·48	8·24		7·63	8·28
2-Desvinyl-γ-formyl-2-methoxycarbonyl-pyrrochlorin-methyl ester	−1·81	−0·31 (α) 0·61 (β) 1·19 (δ) 0·58 (δ)			5·70		6·41	6·02	6·39	6·88	6·61	8·21		7·63	8·29
γ-Cyano-2-desvinyl-2-methoxycarbonylpyrrochlorin methyl ester		−0·33 (α) 0·68 (β) 1·32 (δ) 1·35 (δ)			5·73		6·43	5·35	6·32	6·76	6·49	8·23		7·52	8·36

TABLE 2

Visible spectra of some chlorins and their *meso*-substituted derivatives in chloroform ($\lambda_{max.}$ in mμ; 10^{-3} ε in parentheses)

Chlorin	Soret	Band V	Band IV	Band III	Band II	Band I
Isochlorin-e_4 dimethyl ester	401 (153)	503 (12·6)	529 (3·1)	557 (2·0)	606 (3·5)	659 (39·3)
Chlorin-e_6 trimethyl ester	404 (176)	502 (12·9)	532 (5·7)	559 (3·1)	610 (5·0)	664 (42·4)
2-Desvinyl-2-(3-oxoprop-1-enyl)isochlorin-e_4 dimethyl ester	424 (80)	514 (8·6)	551 (8·4)	580sh (2·2)	641 (4·0)	691 (40·6)
2-Desvinyl-γ-formyl-2-methoxycarbonylpyrrochlorin dimethyl ester	416 (156)	505 (10·2)	541 (11·9)	586sh (1·9)	648 (7·3)	699 (30·1)
γ-Cyano-2-desvinyl-2-methoxycarbonylpyrrochlorin dimethyl ester	403 (101)	500 (8·0)	533 (5·7)	580sh (1·5)	630 (3·3)	683 (40·4)
Rhodin-g_7 trimethyl ester	430 (161)	530 (9·5)	559 (7·6)		600 (5·5)	652 (20·2)
3-Cyano-3-desformylrhodin-g_7 trimethyl ester	421 (204)	524 (11·2)	554 (6·8)		596 (4·5)	647 (25·0)
Purpurin-5 dimethyl ester	410 (112)	507 (7·3)	544 (10·5)		644sh (5·5)	694 (27·2)
2-Desvinyl-2-(3-oxoprop-1-enyl)chlorin-e_6 trimethyl ester	422 (72)	514 (6·4)	552 (8·1)	584sh (2·1)	644sh (4·5)	697 (32·3)
2-Desvinyl-δ-formyl-2-(3-oxoprop-1-enyl)chlorin-e_6 trimethyl ester	430 (78)	525 (5·2)	571 (8·5)		658sh (6·5)	729 (33·4)

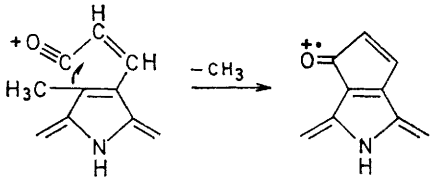
chlorin-e_6 trimethyl ester (IIIb). The n.m.r. spectra of the diformylated compound was similar to that of the monoformylated compound but showed the presence of only two methine protons and a single signal at τ −1·59, assigned to a δ-formyl substituent. It is thus assigned the structure 2-desvinyl-δ-formyl-2-(3-oxoprop-1-enyl)-chlorin-e_6 trimethyl ester (IIIc). The appearance of a base peak of M − 16 units for compound (IIIb) suggests

TABLE 3

Effect of formylation on the spectra of metal complexes of protoporphyrin IX dimethyl ester

	$\lambda_{max.}$ ($CHCl_3$)	
Complex	Before formylation	After formylation
Mn^{III}	474, 565, 597	480, 570, 615
Co^{II}	528, 562	590
Fe^{III}	512, 540, 638	520, 557, 656
Cu^{II}	534, 574	554, 597
Ni^{II}	523, 561	543, 585, 629

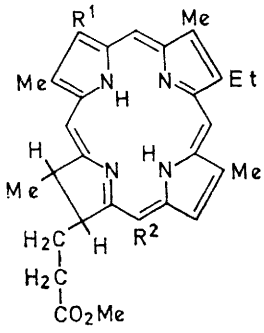
the expulsion of methane. A possible mechanism for this process is the formation of a fused five-membered ring ion (IV) as shown. In the mass spectrum of



(IV) (M − 16)

diformylchlorin-e_6 trimethyl ester (IIIc) the molecular ion is almost absent, the base peak being of M − 45 units. This peak presumably arises from loss of formyl from an ion analogous to that shown. The iron(III) complex of isochlorin-e_4 dimethyl ester (Va) reacted similarly to chlorin-e_6 trimethyl ester on formylation. Only one product was isolated, and was assigned the structure 2-desvinyl-2-(3-oxoprop-1-enyl)isochlorin-e_4 dimethyl ester (Vb) on the basis of similar criteria to those used for the products from chlorin-e_6 dimethyl ester.

In order to compare the spectroscopic properties of these compounds with the properties of other formylchlorins, oxidation of chlorin-e_6 and isochlorin-e_4 with permanganate was attempted. Under the conditions used by Fischer [12] chlorin-e_6 yielded purpurin-5 dimethyl ester (IIId) in low yield. Isochlorin-e_4, on the other hand, did not yield γ-formylpyrrochlorin methyl ester (Vc) but a product which did not show a vinyl proton signal in its n.m.r. spectrum. The presence of a signal in a position similar to that of the 6-methoxy-group of chlorin-e_6 trimethyl ester suggested the structure 2-desvinyl-γ-formyl-2-methoxycarbonylpyrrochlorin methyl ester (Vd). The position of the aldehyde proton signals in the δ- and γ-*meso*-formylchlorins varied from −1·81 to −1·15; this is about 1 p.p.m. to higher field than in the *meso*-formylporphyrins, reflecting the lower deshielding effect of the dihydropyrrole ring of the chlorins. Rhodin-g_7 trimethyl ester (IIIe), which has a β-formyl substituent, showed an aldehyde proton signal at only slightly higher field (−1·05 p.p.m.). On the other hand the 3-oxoprop-1-enylchlorins showed aldehyde proton signals at considerably higher field (*ca.* 0·1 p.p.m.), owing to their greater separation from the deshielding influence of the chlorin ring current.



(V)

	R^1	R^2
a;	$CH=CH_2$	$CH_2{\cdot}CO_2Me$
b;	$CH=CH{-}CHO$	$CH_2{\cdot}CO_2Me$
c;	$CH=CH_2$	CHO
d;	CO_2Me	CHO
e;	CO_2Me	CN

The effect of cyano-substituents on the spectra of chlorins was also investigated because of the postulation of a *meso*-cyanochlorin structure for the iron-free derivative of pseudohaem,[13] a compound previously thought to be an intermediate in bile pigment formation. This product had an unusual visible spectrum characterised by a very low band I. Attempted conversion of purpurin-5 dimethyl ester (IIId) into an oxime resulted in deformylation to yield rhodochlorin dimethyl ester (IIIf). This reaction paralleled the ready loss of carbon monoxide from the molecular ion seen in the mass spectrum and reflects steric crowding around the γ-position. 2-Desvinyl-γ-formyl-2-methoxycarbonylpyrrochlorin methyl ester was, however, easily converted into the oxime, which was dehydrated to give the analogous γ-cyanochlorin (Ve). Rhodin-g_7 trimethyl ester was also converted into the 3-cyanochlorin (IIIg) by a similar procedure. Both compounds showed conjugated C≡N absorption in their i.r. spectra at 2210 $cm.^{-1}$. Both cyanochlorins showed visible absorption bands of fairly normal intensity ratios. However, the absence of an adjacent β-substituent in the γ-cyanochlorin (Ve) may cause the disappearance of steric effects which could well result in the lowering of band I in the pseudohaem derivative. The introduction of formyl substituents into the chlorin ring system at the vinyl terminal methylene, γ-, or δ-positions caused bathochromic shifts in the visible absorption spectrum of about 30 mμ in band I. A similar shift has been observed for 2-desvinyl-2-formylchlorin-e_6 trimethyl ester,[14] which has a spectrum almost

[12] H. Fischer and K. Kahr, *Annalen*, 1937, **531**, 209.
[13] D. B. Morell and A. W. Nichol, *J. Chem. Soc.* (*C*), 1969, 517.
[14] H. Fischer and H. Walter, *Annalen*, **549**, 44.

identical with that of 2-desvinyl-2-(3-oxoprop-1-enyl)-chlorin-e_6 trimethyl ester. As in the porphyrin series a cyano-substituent at the 2-position (as in 2-cyano-2-desvinylchlorin-e_6 [14]) or at the γ-position causes a slightly smaller bathochromic shift than the formyl group. In contrast to the porphyrin series, where introduction of a second electrophilic substituent causes a much smaller shift, a second formyl group in diformylchlorin-e_6 (IIIc) caused a shift in the position of band I almost identical with that caused by the first.

Rhodin-g_7, with a formyl substituent at the 3-position, on the pyrrole ring opposite to the dihydropyrrole ring, shows a shift in band I of 12 mμ to shorter wavelength in comparison with chlorin-e_6. The difference between the spectra of rhodin-g_7 and chlorins with electrophilic substituents on the pyrrole ring adjacent the reduced ring has been partially explained by Fischer,[14] in terms of a difference in the structure of the rings involved. A more complete explanation may, however, be found in a recent suggestion [15] that the visible bands of the porphyrins are due to $n \rightarrow \pi$ transitions of the non-bonded nitrogen electrons. If the nitrogen atom of the reduced ring and that opposite to it are in the non-protonated form the shift to shorter wavelengths could be due to an increase in the separation of energy levels of the lone pair of electrons of ring II, due to the inductive effect of the 3-formyl group.[16] Rapid tautomerism,[17] or sharing of the two inner protons between all four nitrogen atoms,[18] which may occur in the porphyrin series seems less likely in the chlorin series since protonation of the nitrogen atom of the dihydropyrrole ring would necessitate a contribution from high-energy charge-separated resonance structures to maintain the macro-ring conjugation.

The preparation of iron–chlorin–globin complexes of some of the compounds prepared here and a comparison of the spectra of these complexes with myeloperoxidase will be reported elsewhere.

EXPERIMENTAL

M.p.s were determined with a Reichert hot-stage microscope. I.r. spectra were determined with a Perkin-Elmer 337 spectrometer; n.m.r. spectra were determined with a Varian A60 spectrometer. An A.E.I. MS9 instrument was used for mass spectrometry and a Perkin-Elmer 137 u.v. spectrophotometer for the determination of visible spectra. Schuchardt alumina (activity 1) was used for chromatography. Chloroform was destabilised before use and dried over anhydrous potassium carbonate. Analyses were performed by the Australian Microanalytical Service, C.S.I.R.O., Melbourne.

Protohaemin, deuterohaem, and mesohaemin dimethyl esters were prepared by the methods given by Falk.[19] Chlorin-e_6, rhodin-g_7, and isochlorin-e_4 methyl esters were prepared from a methanol extract of milled lucern by the methods given by Fischer and Orth.[20]

Formylation of Transition Metal Complexes of Protoporphyrin IX Dimethyl Ester.—Protoporphyrin IX dimethyl ester (0·001 g.) was converted into the metal complexes shown in Table 3 by standard methods.[19] To a solution of the complex in dry chloroform (5 ml.) was added a mixture of dimethylformamide (0·3 ml.) and phosphoryl chloride (0·2 ml.), and the resulting mixture was refluxed for 1 hr. Saturated sodium acetate solution (5 ml.) was added, and the the mixture was refluxed for a further 1 hr. The aqueous phase was acidified, the product was extracted into chloroform, and its spectrum was determined (see Table 3).

Formylation of Deuterohaemin Dimethyl Ester.—Deuterohaemin dimethyl ester (1·0 g.) in dimethylformamide (20 ml.) was treated with a mixture of dimethylformamide (10 ml.) and phosphoryl chloride (13·7 ml.). The mixture was heated on a boiling water bath for 2 hr., poured with caution into saturated aqueous sodium acetate (500 ml.), and heated for a further 1 hr. The product was acidified with hydrochloric acid, and the precipitate was filtered off and warmed with acetic acid (2 l.). Undissolved material was filtered off, dissolved in pyridine (20 ml.), and added to the acetic acid filtrate. A saturated solution of iron(II) sulphate in hydrochloric acid (10N; 40 ml.) was added under nitrogen. The solution was set aside for 1 hr., during which time it was agitated with nitrogen bubbles. Saturated sodium acetate (500 ml.) was then added. The products were transferred in small portions into ether and acetic acid was removed by washing with water. After esterification of a small amount of undissolved material with diazomethane the products were chromatographed on alumina in chloroform. The first band (red) contained a little deuteroporphyrin dimethyl ester; the second (red-violet) yielded β-formyldeuteroporphyrin IX dimethyl ester which crystallised as dark needles (0·145 g.), m.p. 181—185° (from chloroform–methanol) (Found: C, 69·55; H, 6·0; N, 9·8. Calc. for $C_{33}H_{34}N_4O_5$: C, 70·0; H, 6·0; N, 9·9%), $\lambda_{max.}$ ($CHCl_3$) 414 (ε 137,000), 516 (6390), 559 (7850), 582 (4800), and 649 (6400)mμ, $\lambda_{max.}$ (HCO_2H) 411 (231,000), 566 (7540), 619 (6440) mμ, $\nu_{max.}$ (Nujol) 1730 (ester C=O), 1665 (aldehyde C=O) cm.$^{-1}$, τ (CF_3CO_2D) −2·47 (1H, β-CHO), −1·12 (1H, γ-methine), −0·81 (1H, α-methine), −0·68 (1H, δ-methine), 0·24 (1H, 4-proton), 0·67 (1H, 2-proton), 5·42 (4H, m, 1-CH_2 of propionyls), 6·16 (3H, OMe), 6·25 (6H, OMe and ring Me), 6·30 (6H, ring Me), 6·60 (3H, ring Me), and 6·76 (4H, m, 2-CH_2 of propionyls), *m/e* 566 (100%), 538 (78), 535 (9), 512 (m*, 566 ⟶ 538), 507 (8), 493 (17), 479 (20), 465 (38), 452 (12), 438 (4), and 391 (14).

A third band (green), eluted from the column with 1% methanol–chloroform, yielded 2,β-diformyldeuteroporphyrin IX dimethyl ester as dark needles (0·193 g.), m.p. 228—230° (from chloroform–methanol) (Found: C, 68·4; H, 5·8; N, 9·6. Calc. for $C_{34}H_{34}N_4O_6$: C, 68·7; H, 5·7; N, 9·4%), $\lambda_{max.}$ ($CHCl_3$) 426 (ε 180,700), 533 (10,520), 575 (18,900), 594 (14,620), and 654 (7630) mμ, $\lambda_{max.}$ (HCO_2H) 424 (326,000), 577 (13,800), and 629 (13,600) mμ, $\nu_{max.}$ (Nujol) 3300 (NH), 1730 (ester C=O), and 1670 (aldehyde C=O) cm.$^{-1}$, τ (CF_3CO_2D) −2·38 (1H, β-CHO), −1·43 (1H,

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2-CHO), −1·32 (1H, γ-methine), −1·03 (1H, α-methine), −0·93 (1H, δ-methine), 5·57 (4H, m, 1-CH_2 of propionyls), 5·99 (3H, ring Me), 6·13 (3H, OMe), 6·26 (3H, OMe), 6·33 (6H, ring Me), 6·65 (3H, ring Me), and 6·83 (4H, m, 2-CH_2 of propionyls), *m/e* 594 (48%), 566 (100), 538 (21), 521 (7), 507 (20), 493 (49), 479 (13), 466 (14), 420 (4), and 391 (8).

Formylation of Protohaemin Dimethyl Ester.—Protohaemin dimethyl ester (1 g.) dissolved in dimethylformamide (10 ml.) was treated with a mixture of dimethylformamide (10 ml.) and phosphoryl chloride (13·7 ml.). The mixture was heated on a boiling water bath for 3 min., poured into saturated sodium acetate solution (500 ml.), and set aside at room temperature for 2 hr. Iron was removed as before and the porphyrin dimethyl esters were chromatographed on alumina with 0·5% methanol–chloroform. The first band yielded 2(or 4)-(3-*oxoprop*-1-*enyl*)-4(or 2)-*vinyldeuteroporphyrin IX dimethyl ester* as red-brown needles (0·065 g.), m.p. 168—171° (from chloroform–methanol) (Found: C, 71·5; H, 6·4; N, 8·9. $C_{37}H_{38}N_4O_5$ requires C, 71·9; H, 6·2; N, 9·1%), $\lambda_{max.}$ ($CHCl_3$) 426 (ε 95,400), 517 (6240), 560 (11,500), 585 (7300), and 648 (1540) mμ, $\lambda_{max.}$ (HCO_2H) 414 (194,000), 561 (9670), and 611 (4950) mμ, $\nu_{max.}$ (Nujol) 3300 (N–H), 1725 (ester C=O), and 1660 (aldehyde C=O) $cm.^{-1}$, τ (CF_3CO_2D) −1·12 (2H, methine), −1·08 (2H, methine), 0·36 [0·3H, d, CHO of *cis*-(3-oxoprop-1-enyl)], 0·61 [0·7H, d, CHO of *trans*-(3-oxoprop-1-enyl)], 2·28 (3H, m, CH of vinyl and CH of propenyls), 3·45 (2H, m, CH of vinyl), 5·30 (4H, m, 1-CH_2 of propionyls), 6·01 (3H, ring Me), 6·20 (15H, OMe and ring Me), and 6·68 (4H, m, 2-CH_2 of propionyls), *m/e* 618 (100%), 602 (16), 559 (23), and 545 (43).

A second band, eluted from the column with 1% methanol –chloroform, yielded 2,4-*bis*-(3-*oxoprop*-1-*enyl*)*deuteroporphyrin IX dimethyl ester* as violet-brown needles (0·175 g.), m.p. 229—234° (from chloroform–methanol) (Found: C, 70·4; H, 6·0; N, 8·7. $C_{38}H_{38}N_4O_6$ requires C, 70·6; H, 5·9; N, 8·7%), $\lambda_{max.}$ ($CHCl_3$) 443 (ε 80,500), 527 (9300), 565 (9750), 595 (5540), and 651 (3760) mμ, $\lambda_{max.}$ (HCO_2H) 424 (165,000), 568 (11,420), and 615 (4960) mμ, $\nu_{max.}$ (Nujol) 3300 (N–H), 1730 (ester C=O), and 1670 (aldehyde C=O) $cm.^{-1}$, τ (CF_3CO_2D) −1·24 (1H, γ-methine), −1·20 and −1·18 (2H, α- and β-methines), −1·08 (1H, δ-methine), 0·36 (0·8H, d, *cis*-CHO), 0·65 (1·2H, d, *trans*-CHO), 1·52 (2H, m, 1-CH of oxopropenyls), 2·20 (2H, m, 2-CH of oxopropenyls), 5·35 (4H, m, 1-CH_2 of propionyls), 6·03 (6H, 1- and 3-Me), 6·20 (12H, OMe and ring Me), and 6·70 (4H, m, 2-CH_2 of propionyls), *m/e* 646 (100%), 630 (29), 615 (42), 587 (12), and 573 (15).

2,4-*Bis*-(3-*hydroxyprop*-1-*enyl*)*deuteroporphyrin IX Dimethyl Ester.*—2,4-Bis-(3-oxoprop-1-enyl)deuteroporphyrin IX dimethyl ester (0·075 g.) in chloroform–methanol (1 : 1 v/v; 100 ml.) was treated with sodium borohydride (0·5 g.). After being stirred for 10 min. the solution was washed with water (×3) and the precipitated product was redissolved in the chloroform layer by addition of methanol and crystallised from chloroform–methanol after evaporation. 2,4-*Bis*-(3-*hydroxyprop*-1-*enyl*)*deuteroporphyrin IX dimethyl ester* was obtained as red brown needles (0·055 g.), m.p. 300° (Found: C, 69·9; H, 6·35; N, 8·4. $C_{38}H_{42}N_4O_6$ requires C, 70·2; H, 6·45; N, 8·6%), $\lambda_{max.}$ ($CHCl_3$) 409 (ε 148,000), 512 (12,920), 547 (11,280), 584 (6600), 636 (4780) mμ, $\lambda_{max.}$ (HCO_2H) 409 (258,000), 561 (16,500), and 607 (7100) mμ, $\nu_{max.}$ (Nujol) 3250br (NH and OH) and 1730 (ester C=O) $cm.^{-1}$, τ (CF_3CO_2D) −1·16 (1H, γ-methine), −1·13 (1H, α-methine) −1·07 (2H, β- and δ-methine), 1·70 (2H, m, 1-CH of propenyls), 2·91 (2H, m, 2-CH of propenyls), 4·39 (1·5H, d, *J* 4 Hz, *cis*-CH_2 of propenyls), 4·93 (2·5H, d, *J* 5 Hz, *trans*-CH_2 of propenyls), 5·31 (4H, m, 1-CH_2 of propionyls), 6·17 (6H, ring Me), 6·19 (6H, ring Me), 6·24 (6H, OMe), and 6·69 (4H, m, 2-CH_2 of propionyls), *m/e* 650 (3%), 648 (4), 632 (22), 614 (100), 606 (1), 602 (5), 592 (27), 566 (8), 555 (9), 541 (16), and 529 (35).

β-*Formylmesoporphyrin IX Dimethyl Ester.*—Mesohaemin dimethyl ester (1 g.) in dry chloroform (150 ml.) was refluxed with a mixture of dimethylformamide (10 ml.) and phosphoryl chloride (13·5 ml.) for 2 hr. A small sample was removed, heated with saturated sodium acetate, and treated with pyridine and sodium dithionite. Examination with a hand spectroscope showed only the spectrum of pyridine mesohaemochrome ($\lambda_{max.}$ 518 and 547 mμ). The chloroform was distilled from the original reaction mixture and the residue was heated on a boiling water bath for 3 hr. A pyridine haemochrome, prepared after this period, showed a broad band at 603 mμ. Iron was removed as described for the formylation of deuterohaemin dimethyl ester. The porphyrins were chromatographed on alumina in chloroform. The first band yielded mesoporphyrin dimethyl ester (0·22 g.) as red-brown needles, m.p. 210—212° (from chloroform–methanol). Continued elution with chloroform gave a red-violet band which yielded β-*formylmesoporphyrin IX dimethyl ester* (0·15 g.) as long violet-brown needles, m.p. 201—205° (from chloroform–methanol) (Found: C, 71·2; H, 6·7; N, 9·0. $C_{37}H_{42}N_4O_5$ requires C, 71·4; H, 6·8; N, 9·0%), $\lambda_{max.}$ ($CHCl_3$) 410 (ε 122,000), 509 (6250), 543 (5180), 582 (5240), and 631 (3100) mμ, $\lambda_{max.}$ (HCO_2H) 418 (164,000), 571 (6900), and 625 (5150) mμ, $\nu_{max.}$ (Nujol) 1730 (ester C=O, and 1690 (aldehyde C=O) $cm.^{-1}$, τ (CF_3CO_2D) −2·45 (1H, CHO), −1·01 (1H, γ-methine), −0·75 (2H, α- and δ-methine), 5·63 (4H, m, 1-CH_2 of propionyls), 5·89 (2H, q, CH_2 of 2-ethyl), 6·14 (3H, OMe), 6·26 (3H, OMe), 6·32 (3H, Me), 6·42 (2H, q, CH_2 of 4-ethyl), 6·65 (3H, Me), 6·78 (3H, Me), 6·85 (4H, m, 2-CH_2 of propionyls), 6·88 (3H, Me), 8·42 (3H, t, Me of 2-ethyl), and 8·70 (3H, t, Me of 4-ethyl), *m/e* 622 (35%), 594 (100), 579 (19), 563 (8), 535 (11), 521 (42), 457 (*m**, 594 ⟶ 521), and 433 (5).

2-*Desvinyl*-2-(3-*oxoprop*-1-*enyl*)*isochlorin-e*$_4$ *Dimethyl Ester.*—Isochlorin-e_4 dimethyl ester (0·1 g.) in pyridine (1 ml.) was added to a mixture of glacial acetic acid (10 ml.) and saturated aqueous iron(II) sulphate (0·5 ml.). The solution was heated at 100° until the absorption band at 659 mμ (hand spectroscope) was replaced by a band at 600 mμ (*ca.* 10 min.). The solution was poured into chloroform (20 ml.) and washed with dilute hydrochloric acid (×3). The solvent was evaporated off and the residue in dimethylformamide (1 ml.) was heated on a boiling water bath; a mixture of dimethylformamide (1 ml.) and phosphoryl chloride (1 ml.) was added. Heating was continued for 30 sec., after which the mixture was poured into saturated aqueous sodium acetate (100 ml.) and heated at 80° for 1 hr. The precipitate was extracted into chloroform (×2) and the extract was then washed with water (×2) and dried. The solvent was evaporated off and the residue was dissolved in acetic acid (50 ml.); a saturated solution of iron(II) sulphate in 10N-hydrochloric acid (1 ml.) was added under nitrogen. After 10 min. the solution was poured into chloroform; the resulting solution was washed with water (×3), dried, and evaporated. The residue was chromatographed on alumina in chloroform. The first band (green) yielded a little unchanged isochlorin-e_4 dimethyl ester. The second (violet-brown) yielded 2-*desvinyl*-2-(3-*oxoprop*-

1-*enyl*)*isochlorin-e*$_4$ *dimethyl ester* (0·035 g.) as violet-brown needles, m.p. 266—267° (from chloroform–methanol) (Found: C, 70·8; H, 6·6; N, 9·0. $C_{36}H_{40}N_4O_5$ requires C, 71·1; H, 6·6; N, 9·2%), $\nu_{max.}$ (Nujol) 3330 (N–H), 1740, 1720 (ester C=O), 1670 (aldehyde C=O) cm.$^{-1}$, *m/e* 608 (100%), 593 (4), 592 (4), 580 (12), 554 (*m**, 608 ⟶ 580), 549 (9), 535 (2), 521 (20), 497 (*m**, 608 ⟶ 549), 493 (4), 461 (3), 448 (3), 447 (*m**, 608 ⟶ 521), 433 (4), 419 (5), 405 (3), and 390 (2).

Formylation of Chlorin-e$_6$ *Trimethyl Ester*.—Chlorin-e$_6$ trimethyl ester (0·4 g.) was converted into the iron(III) complex ($\lambda_{max.}$ 610 mμ) and formylated as in the formylation of isochlorin-e$_4$ dimethyl ester except that the reaction was allowed to proceed for 90 sec. After removal of iron the products were chromatographed on alumina with 0·1% methanol–chloroform as eluant. The first band (violet-brown) yielded 2-*desvinyl*-2-(3-*oxoprop*-1-*enyl*)*chlorin-e*$_6$ *trimethyl ester* (0·03 g.) as brown needles, m.p. 208—209° (from chloroform–methanol) (Found: C, 68·2; H, 6·4; N, 8·5. $C_{38}H_{42}N_4O_7$ requires C, 68·5, H, 6·3; N, 8·4%), $\nu_{max.}$ (Nujol) 3310 (N–H), 1730, 1720 (ester C=O), and 1670 (aldehyde C=O) cm.$^{-1}$, *m/e* 666 (21%), 650 (100), 635 (4), 619 (21), 605 (17), 589 (14), 576 (42), 561 (4), 547 (3), 519 (4), 512 (*m**, 650 ⟶ 576), and 507 (4).

A second band (violet) yielded 2-*desformyl*-δ-*formyl*-2-(3-*oxoprop*-1-*enyl*)*chlorin-e*$_6$ *trimethyl ester* (0·12 g.) as violet needles, m.p. 143—148° (from chloroform–methanol) (Found: C, 67·7; H, 6·2; N, 8·1. $C_{39}H_{42}N_4O_8$ requires C, 67·5; H, 6·05; N, 8·1%), $\nu_{max.}$ (Nujol) 3320 (N–H), 1730 (ester C=O), and 1670 (aldehyde C=O) cm.$^{-1}$, *m/e* 694 (2%), 678 (25), 667 (12), 649 (100), 635 (11), 19 (27), 605 (22), 589 (11), 576 (38), 561 (5), 547 (4), and 514 (*m**, 649 ⟶ 576).

Purpurin-5 Dimethyl Ester.—This was prepared by oxidation of chlorin-e$_6$ (0·7 g.) with potassium permanganate as described by Fischer and Kahr.[12] The esterified products separated by chromatography on an alumina column in chloroform. The first band to be eluted contained purpurin-5 dimethyl ester (0·05 g.), which crystallised from methanol–chloroform as violet plates, m.p. 191—193° (lit.,[12] 194°) (Found: C, 70·35; H, 6·5; N, 9·2. Calc. for $C_{35}H_{38}N_4O_5$: C, 70·7; H, 6·4; N, 9·4%), $\nu_{max.}$ 3320 (N–H), 1730 (ester C=O), and 1670 (aldehyde C=O) cm.$^{-1}$, *m/e* 594 (10%), 564 (100), 549 (5), 522 (8), 491 (9), and 478 (9). Further elution gave a small amount of product with $\lambda_{max.}$ ($CHCl_3$) 413, 516, 554, 594, 661, and 716 mμ (I > IV > V > II > III).

Deformylation of Purpurin-5 Dimethyl Ester.—To purpurin-5 dimethyl ester (0·02 g.) in 70% aqueous pyridine (2 ml.) hydroxylamine hydrochloride (0·05 g.) was added and the mixture was heated at 60° for 30 min. It was poured into ether (20 ml.), and pyridine was removed by shaking with 5% hydrochloric acid (×3). The product was dried and evaporated, and the residue was chromatographed on alumina in chloroform. The first band (green) contained most of the product, which crystallised from chloroform–methanol as green needles (0·005 g.) of rhodochlorin dimethyl ester, m.p. 210—212° (lit.,[21] 207°) (Found: C, 72·1; H, 6·5. Calc. for $C_{34}H_{38}N_4O_4$: C, 72·1; H, 6·7%), M^+ 566, $\lambda_{max.}$ ($CHCl_3$) 408, 500, 528, 554, 608, and 665 mμ (I > V > IV > II > III) (lit.,[21] 496, 528, 558, 609, and 666 mμ).

2-*Desvinyl*-γ-*formyl*-2-*methoxycarbonylpyrrochlorin Methyl Ester*.—Isochlorin-e$_4$ dimethyl ester (0·3 g.) was dissolved in pyridine (4 ml.) and treated with boiling 30% methanolic potassium hydroxide (5 ml.). Boiling was continued for 4 min. and the mixture was poured into an excess of dilute hydrochloric acid, adjusted to pH 5·0 with sodium hydrogen carbonate, and extracted with ether (×3). The ether extract was evaporated to dryness and dissolved in pyridine (5 ml.). A solution of potassium permanganate (0·12 g.) in pyridine (50 ml.) was added and the mixture was stirred for 1 hr. and poured into water (100 ml.). It was adjusted to pH 3·0 with hydrochloric acid and extracted with chloroform (×3). The extract was washed with water (×4) and treated with ethereal diazomethane [from nitrosomethylurea (0·5 g.)], and after 5 min. acetic acid (1 ml.) was added and the solution was evaporated to dryness. The residue was chromatographed on alumina in chloroform. The first band (violet-brown) contained the major product, which yielded violet needles (0·054 g.), m.p. 183—185°, of 2-*desvinyl*-γ-*formyl*-2-*methoxycarbonylpyrrochlorin methyl ester* (from chloroform–methanol) (Found: C, 69·8; H, 6·2; N, 9·5. $C_{33}H_{36}N_4O_5$ requires C, 69·7; H, 6·3; N, 9·9%), $\nu_{max.}$ (Nujol) 3300 (N–H), 1725 (ester C=O), 1700 (ester C=O), 1655 (aldehyde C=O) cm.$^{-1}$, *m/e* 568 (92%), 554 (69), 538 (57), 524 (21), 509 (15), 481 (100), and 451 (85).

γ-*Cyano*-2-*desvinyl*-2-*methoxycarbonylpyrrochlorin Methyl Ester*.—To 2-desvinyl-γ-formyl-2-methoxycarbonylpyrrochlorin methyl ester (0·06 g.) in aqueous pyridine (70%; 10 ml.) hydroxylamine hydrochloride (0·1 g.) was added, and the mixture was heated on a boiling water bath for 30 min. The solution was poured into chloroform (20 ml.) and washed with water (×3). The chloroform solution showed absorption maxima at 505, 545, 625, and 675 mμ. It was evaporated to dryness and the residue was dissolved in acetic anhydride (10 ml.), refluxed for 20 min., and evaporated to dryness after the addition of water (10 ml.). The residue was evaporated with toluene (10 ml.) to remove the last traces of acetic acid, dissolved in chloroform, and treated with a solution of diazomethane [from nitrosomethyl urea (0·2 g.)] in ether (5 ml.). The solvent was evaporated off and the residue was crystallised from chloroform–methanol to yield γ-*cyano*-2-*desvinyl*-2-*methoxycarbonylpyrrochlorin methyl ester* (0·032 g.) as brown needles, m.p. 178—179° (Found: C, 70·0; H, 6·2; N, 12·4. $C_{33}H_{35}N_5O_4$ requires C, 70·1; H, 6·2; N, 12·4%), $\nu_{max.}$ (Nujol) 3330 (N–H), 2205 (C≡N), 1730, and 1705 (ester C=O) cm.$^{-1}$, *m/e* 565 (100%), 550 (6), 533 (46), 521 (5), 490 (7), 478 (44), 445 (19), and 404 (*m**, 565 ⟶ 478).

3-*Cyano*-3-*desformylrhodin-g*$_7$ *Trimethyl Ester*.—Rhodin-g$_7$ trimethyl ester (0·2 g.) was dissolved in aqueous pyridine (70%; 75 ml.), hydroxylamine hydrochloride (0·2 g.) was added, and the mixture was heated on a boiling water bath for 5 min. It was then poured into chloroform (100 ml.) and washed with 5% hydrochloric acid until the washings were acid to litmus. The solution showed maxima at 514, 554, 607, and 664 mμ. It was evaporated and the residue, dissolved in acetic anhydride (20 ml.), was refluxed for 30 min. The solution was poured into water and stirred for 1 hr., and the precipitated solid was filtered off and dissolved in chloroform. It was washed with water (×3) and ethereal diazomethane [from nitrosomethyl urea (0·5 g.)] was added. The solution was set aside for 5 min., treated with acetic acid (0·1 ml.), and evaporated to dryness. The residue was chromatographed on alumina in chloroform. The first band (violet) yielded 3-*cyano*-3-*desformylrhodin-g*$_7$ *trimethyl ester* (0·07 g.) as violet needles m.p. 251—252° (from chloroform–methanol) (Found: C, 68·15; H, 6·1; N, 10·6. $C_{37}H_{39}N_5O_6$ requires C, 68·4; H, 6·0; N, 10·8%),

[21] J. B. Conant, J. F. Hyde, W. W. Moyer, and E. M. Dietz, *J. Amer. Chem. Soc.*, 1931, **53**, 359.

$\nu_{max.}$ (Nujol) 3300 (N–H), 2210 (C≡N), 1730, and 1700 (ester C=O) cm.$^{-1}$, *m/e* 649 (100%), 634 (16), 618 (5), 590 (25), 586 (*m**, 649 ⟶ 618), 576 (17), 564 (10), 562 (8), 558 (5), 537 (*m**, 649 ⟶ 590), 530 (4), 516 (3), 514 (*m**, 649 ⟶ 576), 502 (10), and 490 (27).

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